Anthraquinone, Cyclopentanone, and Naphthoquinone Derivatives from the Sea Fan-Derived Fungi *Fusarium* spp. PSU-F14 and PSU-F135

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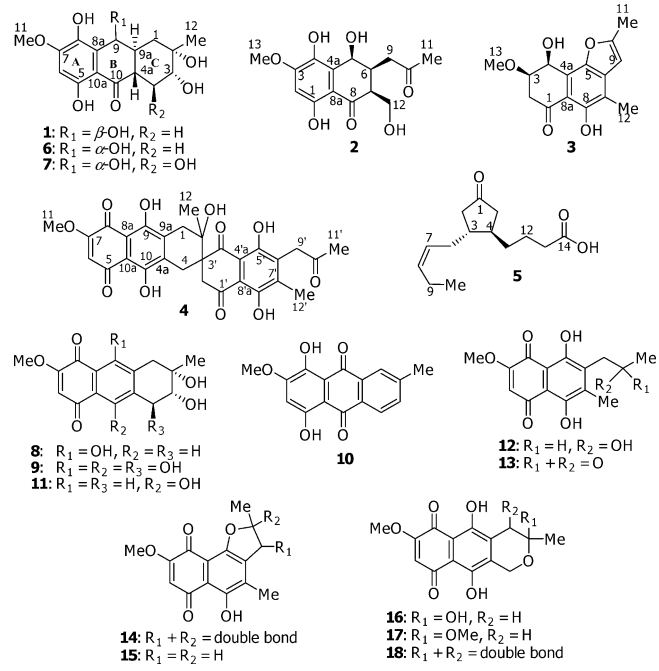
Five new metabolites, fusaranthraquinone (**1**), fusarnaphthoquinones A−C (**2**−**4**), and fusarone (**5**), were isolated from the sea fan-derived fungi *Fusarium* spp. PSU-F14 and PSU-F135 along with 18 known compounds. The structures were elucidated on the basis of spectroscopic evidence. Their antibacterial, antifungal, antimycobacterial, antimalarial, and cytotoxic activities were examined.

Fungi in the genus *Fusarium* with the capability of producing various bioactive metabolites have drawn attention from many chemists to investigate their metabolites. They have produced a broad range of secondary metabolites that show various biological activities. Some of them are the antifungal fungerin,[1] the antibacterial and cytotoxic neomangicols,[2] and the anti-*Helicobacter pylori* methylsulochrin.[3] We have recently reported the investigation of sea fan-derived fungi that yielded the antibacterial nigrospoxydon A[4] and nigrosporapyrone A.[5] In this paper, we describe the isolation and structure determination of metabolites from two sea fan-derived fungi, *Fusarium* spp. PSU-F14 and PSU-F135. One new octahydroanthraquinone, fusaranthraquinone (**1**), and four new compounds, three hydronaphthoquinones, fusarnaphthoquinones A−C (**2**−**4**), and one cyclopentanone, fusarone (**5**), were obtained from the broth extracts of *Fusarium* spp. PSU-F14 and PSU-F135, respectively. Seven known compounds, 9α-hydroxydihydrodesoxybostrycin (**6**),[6] 9α-hydroxyhalorosellinia A (**7**),[6] nigrosporin A (**8**),[7] bostrycin (**9**),[8] austrocortirubin (**10**),[9] (+)-dehydrovomifoliol,[10] and 2-(2′*S*-hydroxypropyl)-5-methyl-7-hydroxychromone,[11] were also obtained from the broth extract of the fungus PSU-F14, while its mycelial extract contained two additional known compounds, nigrosporin B (**11**)[7] and uridine.[12] Compound **9** was isolated as its 11-acetoxy derivative. Moreover, nine known compounds, aspergillol B,[13] (+)-solaninol (**12**),[14] javanicin (**13**),[15] 5-hydroxy-8-methoxy-2,4-dimethylnaphtho[1,2-*b*]furan-6,9-dione (**14**),[14] 2,3-dihydro-5-hydroxy-8-methoxy-2,4-dimethylnaphtho[1,2-*b*]furan-6,9-dione (**15**),[14] fusarubin (**16**),[16] methyl ether fusarubin (**17**),[17] anhydrofusarubin (**18**),[16] and tyrosol,[18] were obtained from the broth extract of *Fusarium* sp. PSU-F135. Their antibacterial, antifungal, antimalarial, antimycobacterial, and cytotoxic activities were evaluated.



Results and Discussion

The marine-derived fungi PSU-F14 and PSU-F135, isolated from a gorgonian sea fan (*Annella* sp.), were identified on the basis of their morphological and molecular characteristics as *Fusarium* spp. All compounds were isolated using chromatographic techniques, and their structures were elucidated by spectroscopic data (IR, UV, NMR, and MS). The relative configuration was assigned according to NOEDIFF results. The absolute configurations of the known compounds were determined by comparison of their specific rotations with those previously reported in the literature (Supporting Information).

Fusaranthraquinone (**1**) was obtained as a colorless gum with the molecular formula $C_{16}H_{20}O_7$ from HREIMS. The UV absorption bands at 242, 280, and 353 nm revealed the presence of a conjugated carbonyl chromophore, while the IR spectrum showed absorption bands for hydroxy and conjugated ketone carbonyl groups at 3417 and 1667 cm^{-1}, respectively. The ^1H NMR and ^{13}C NMR spectra (Table 1) were similar to those of 9α-hydroxydihydrodesoxybostrycin (**6**)[6] with significant differences in the chemical shifts of H_2-1 (**1**: δ_H 1.87, t, J = 11.5 Hz and 1.76, dd, J = 11.5 and 3.5 Hz; **6**: δ_H 2.43, dd, J = 12.6 and 5.1 Hz and 1.34, dd, J = 12.6 and 11.7 Hz) and the coupling constants between H-9 and H-9a [**1**: J = 3.5 Hz, H-9 (δ_H 5.00, t, J = 3.5 Hz) and H-9a (δ_H 2.16, tt, J = 11.5 and 3.5 Hz); **6**: J = 10.2 Hz, H-9 (δ_H 4.83, d, J = 10.2 Hz) and H-9a (δ_H 2.25, m)]. A small coupling constant of 3.5 Hz established the location of H-9 in a pseudoequatorial position, not at the pseudoaxial position as in **6**. Ring C existed in a chair conformation, identical to **6**, according to the large coupling constants (J = 11.5 Hz) between H-4a (δ_H 2.82, td, J = 11.5 and 4.5 Hz) and H-9a

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Table 1. ^{13}C (75 MHz) and ^{1}H (300 MHz) NMR and HMBC Data of Compound **1** in Acetone-d_6

position	δ_C, mult.	δ_H, mult. (J in Hz)	HMBC
1	40.2, CH_2	1.87, t (11.5)	4a, 9, 9a
		1.76, dd (11.5, 3.5)	2, 3, 4a
2-OH	70.5, C	3.21, s	1, 2, 3
3	74.1, CH	3.47, ddd (11.5, 6.5, 4.5)	4
3-OH		3.81, d (6.5)	2, 3, 4
4	29.6, CH_2	2.38, dt (11.5, 4.5)	2, 3, 4a, 9a
		1.56, q (11.5)	2, 3, 4a, 9a
4a	41.5, CH	2.82, td (11.5, 4.5)	1, 3, 4, 9, 9a
5-OH	158.7, C	12.76, s	5, 6, 10a
6	98.8, CH	6.44, s	5, 7, 8, 10a
7	155.0, C		
8	136.0, C		
8a	129.0, C		
9	62.4, CH	5.00, t (3.5)	4a, 8, 8a, 10a
9-OH		4.15, d (3.5)	8a, 9, 9a
9a	38.5, CH	2.16, tt (11.5, 3.5)	4
10	203.0, C		
10a	107.8, C		
11	55.7, CH_3	3.93, s	7
12	26.7, CH_3	1.29, s	1, 2, 3

Table 2. ^{13}C (75 MHz) and ^{1}H (300 MHz) NMR Data of Compounds **2** and **3** in $CDCl_3$

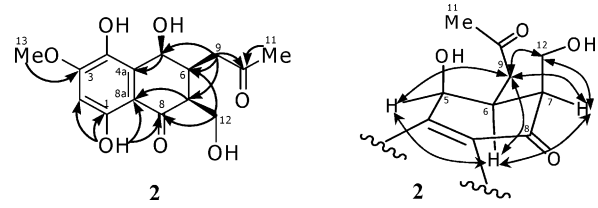
	2		**3**	
position	δ_C, mult.	δ_H, mult. (J in Hz)	δ_C, mult.	δ_H, mult. (J in Hz)
1	158.8, C		201.6, C	
1-OH		11.67, s		
2	99.0, CH	6.37, s	38.4, CH_2	3.16, dd (16.8, 11.1)
				2.89, ddd (16.8, 4.8, 0.9)
3	154.2, C		76.9, CH	3.83, ddd (11.1, 4.8, 3.3)
4	134.5, C		62.3, CH	5.60, brs
4-OH				2.70, d (1.8)
4a	127.6, C		119.6, C	
5	74.6, CH	5.39, s	145.9, C	
6	44.0, CH	2.87, dd (8.1, 5.4)	137.9, C	
7	54.1, CH	2.99, d (5.4)	116.6, C	
8	203.5, C		155.8, C	
8-OH				12.34, s
8a	107.3, C		111.1, C	
9	44.0, CH_2	2.76, dd (17.7, 8.1)	102.6, CH	6.41, q (0.9)
		2.49, dd (17.7, 5.4)		
10	205.8, C		161.4, C	
11	30.3, CH_3	2.20, s	14.6, CH_3	2.50, d (0.9)
12	66.4, CH_2	4.18, dd (9.0, 5.4)	11.6, CH_3	2.33, s
		3.77, d (9.0)		
13	56.4, CH_3	3.91, s	56.6, CH_3	3.55, s

and H-4b (δ_H 1.56, q, J = 11.5 Hz) as well as the NOEDIFF data of H-4a (Figure 1). The NOEDIFF data in Figure 1 further revealed that the relative configurations of all asymmetric carbons in **1**, except C-9, were identical to that of **6**. Consequently, **1** was identified as the 9β epimer of **6**. However, the absolute configuration for **1** remains to be determined.

Fusarnaphthoquinone A (**2**) was obtained as a colorless gum with the molecular formula $C_{15}H_{18}O_7$ by HREIMS. The UV spectrum was similar to that of **1**. A hydroxy absorption band was found at 3330 cm^{-1}, while carbonyl bands were found at 1700 and 1682 cm^{-1}, in the IR spectrum. Compound **2** had a naphthalenone unit (AB ring) related to that of **1** according to similar ^{1}H and ^{13}C NMR data (Table 2) with significant differences in the chemical shifts of C-5, C-6, and C-7 compared to the corresponding carbons in **1** (C-9, C-9a, and C-4a, respectively). The presence of this naphthalenone unit was confirmed by the HMBC correlations depicted in Figure 2. The proton signals for ring C in **1** were replaced by signals for a 2-oxopropyl unit (δ_H 2.49, 1H, dd, J = 17.7 and 5.4 Hz, 2.76, 1H, dd, J = 17.7 and 8.1 Hz, and 2.20, 3H, s) and a hydroxymethyl group (δ_H 4.18, 1H, dd, J = 9.0 and 5.4 Hz and 3.77, 1H, d, J = 9.0 Hz) in **2**. The presence of the 2-oxopropyl unit was supported by the HMBC correlations from both H_2-9 (δ_H 2.76 and 2.49) and H_3-11 (δ_H 2.20) to C-10 (δ_C 205.8). A reduced 1,4-naphthoquinone unit having the 2-oxopropyl and hydroxymethyl substituents at C-6 (δ_C 44.0) and C-7 (δ_C 54.1), respectively, was established on the basis of the HMBC correlations of H_2-9 and H_2-12 (δ_H 4.18 and 3.77) (Figure 2). The substituents at C-5, C-6, and C-7 were located at the same side of the molecule on the basis of signal enhancement of H-5 (δ_H 5.39) and H-7 (δ_H 2.99) upon irradiation of H-6 (δ_H 2.87) as well as that of H_2-9 and H-7 after irradiation of H_2-12 in the NOEDIFF experiment (Figure 2). Therefore, fusarnaphthoquinone A has the structure **2**, with the relative configuration shown.

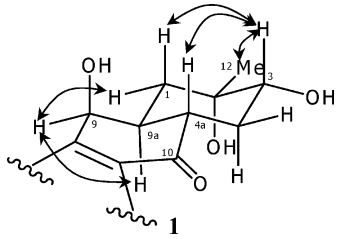
Fusarnaphthoquinone B (**3**) with the molecular formula $C_{15}H_{16}O_5$ by HREIMS was isolated as a red gum. The UV spectrum of **3** exhibited absorption bands at 227, 312, and 360 nm. Hydroxy and ketone carbonyl functionalities were found at 3425 and 1629 cm^{-1}, respectively, in the IR spectrum. The ^{1}H NMR spectrum of **3** (Table 2) was similar to that of **14**, except for the disappearance of a typical signal for a quinone proton (δ_H 6.19, s) in **3** and the appearance of signals typical for two oxymethine protons (δ_H 3.83, ddd, J = 11.1, 4.8, and 3.3 Hz and 5.60, brs) and two nonequivalent methylene protons (δ_H 3.16, dd, J = 16.8 and 11.1 Hz and 2.89, ddd, J = 16.8, 4.8, and 0.9 Hz). In the $^{1}H-^{1}H$ COSY spectrum, one of the oxymethine protons, H-3 (δ_H 3.83), gave cross-peaks with the nonequivalent methylene protons, H_2-2 (δ_H 3.16 and 2.89), and the other oxymethine proton, H-4 (δ_H 5.60). The HMBC correlations H_2-2/C-8a (δ_C 111.1), H-4/C-4a (δ_C 119.6), and H_3-13 (δ_H 3.55)/C-3 (δ_C 76.9) (Figure 3) together with the chemical shifts of C-3 and C-4 (δ_C 62.3) constructed a hydronaphthoquinone skeleton with the methoxy and hydroxy groups at C-3 and C-4, respectively. The signal intensity of H-3 was enhanced when H-4 was irradiated, suggesting their *cis*-relationship. H-3 and H-4 were assigned pseudoaxial and pseudoequatorial orientations, respectively, according to the large J (11.1 Hz) and small J (3.3 Hz) values of H-3 with H-2a (δ_H 3.16) and H-4, respectively. Thus, fusarnaphthoquinone B (**3**) was identified as a new tetrahydronaphthoquinone derivative of **14**. Only the relative configuration is indicated.

Fusarnaphthoquinone C (**4**) was obtained as a red gum. Its molecular formula, $C_{29}H_{26}O_{11}$, by HREIMS indicated that **4** had



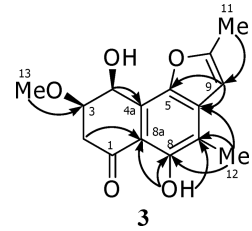
2 **2**

Figure 2. Selected HMBC and NOEDIFF data for **2**.



1

Figure 1. Selected NOEDIFF data for **1**.



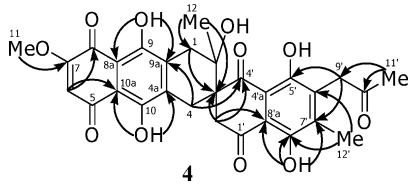
3

Figure 3. Selected HMBC data for **3**.

Table 3. ^{13}C (75 MHz) and ^{1}H (300 MHz) NMR and HMBC Data of Compound **4** in $CDCl_3$

position	δ_C, mult.	δ_H, mult. (*J* in Hz)	HMBC
1	36.8, CH_2	3.12, d (19.8) 3.02, d (19.8)	2, 3, 4a, 9, 9a
2	72.3, C		
3, 3′	54.1, C		
4	32.2, CH_2	3.53, d (19.8) 2.92, d (19.8)	2, 3, 4a, 9a, 2′, 4′
4a	137.6, C		
5	184.3, C		
6	109.7, CH	6.20, s	5, 8, 10a
7	160.7, C		
8	177.8, C		
8a	109.7, C		
9-OH	161.2, C	12.76, s	8a, 9, 9a
9a	134.7, C		
10a	107.9, C		
10-OH	159.6, C	13.17, s	4a, 10, 10a
11	56.8, CH_3	3.94, s	7
12	25.7, CH_3	1.30, s	1, 2, 3
1′	200.4, C		
2′	44.9, CH_2	3.20, d (17.4) 2.88, d (17.4)	2, 3, 4, 1′, 4′, 8′a
4′	203.6, C		
4′a	112.8, C		
5′-OH	154.5, C	12.73, s	4′, 5′, 6′
6′	132.9, C		
7′	139.4, C		
8′-OH	152.8, C	12.24, s	7′, 8′, 8′a
8′a	113.5, C		
9′	41.5, CH_2	3.93, s	5′, 6′, 7′, 10′
10′	203.6, C		
11′	30.0, CH_3	2.30, s	9′, 10′
12′	12.8, CH_3	2.24, s	6′, 7′, 8′

17 degrees of unsaturation. The presence of UV absorption bands at 227, 275, 296, 403, 473, 500, 535, and 571 nm indicated the existence of a 1,4-naphthoquinone chromophore.[14] Furthermore, a hydroxy absorption band was found at 3470 cm^{-1}, while carbonyl ones were found at 1717 and 1644 cm^{-1}, in the IR spectrum. In the ^{1}H and ^{13}C NMR spectra (Table 3), signals for four chelated hydroxy protons [δ_H 12.24 (1H, s), 12.73 (1H, s), 12.76 (1H, s), and 13.17 (1H, s)] and four carbonyl carbons (δ_C 177.8, 184.3, 200.4, and 203.6) suggested the presence of two 1,4-napthoquinone units. Analysis of the HMBC correlations (Table 3, Figure 4) for the left portion of **4** revealed that two chelated hydroxy protons resonating at δ_H 12.76 and 13.17 were attributed to 9-OH and 10-OH, respectively. The olefinic proton (δ_H 6.20) was assigned as H-6 on the basis of its ^{3}J HMBC correlations with C-8 (δ_C 177.8) and C-10a (δ_C 107.9). Irradiation of H-6 enhanced the signal intensity of H_3-11 (δ_H 3.94), indicating the location of the methoxy group at C-7 (δ_C 160.7). The ^{3}J HMBC correlations from H_2-1 (δ_H 3.12 and 3.02) to C-3 (δ_C 54.1) and C-4a (δ_C 137.6) and those from H_2-4 (δ_H 3.53 and 2.92) to C-2 (δ_C 72.3) and C-9a (δ_C 134.7) established a tetrahydroanthraquinone unit. Both methyl (H_3-12, δ_H 1.30) and hydroxy groups were attached to C-2, according to the ^{3}J HMBC correlations of H_3-12 and the chemical shift of C-2. Two remaining chelated hydroxy protons resonating at δ_H 12.24 (8′-OH) and 12.73 (5′-OH) were sequentially placed at C-8′ (δ_C 152.8) and C-5′ (δ_C 154.5). The HMBC correlations from 8′-OH to C-7′ (δ_C 139.4), C-8′, and C-8′a (δ_C 113.5) together with those from the methyl protons (H_3-12′, δ_H 2.24) to C-6′ (δ_C 132.9) and C-8′ attached this methyl group at C-7′. A 2-oxopropyl unit was established and located at C-6′ on the basis of the HMBC correlations of H_2-9′ (δ_H 3.93) and H_3-11′ (δ_H 2.30), as demonstrated in Figure 4. In the NOEDIFF experiment, signal enhancement of 8′-OH and H_2-9′ upon irradiation of H_3-12′ (δ_H 2.24) supported the assigned location of the methyl and 2-oxopropyl groups. In addition, two nonequivalent methylene protons (H_2-2′, δ_H 3.20 and 2.88) displayed ^{3}J HMBC correlations with C-8′a, connecting the methylene group at C-1′ (δ_C 200.4). The HMBC correlations of H_2-2′/C-2, C-3 (C-3′), and C-4′ and those of H_2-4/C-4′ established a spiro skeleton by connecting the dihydronaphthoquinone and tetrahydroanthraquinone at C-3 (C-3′). Therefore, fusarnaphthoquinone C (**4**) was identified as a new spiro dihydronaphthoquinone-tetrahydroanthraquinone derivative.



4

Figure 4. Selected HMBC data for **4**.

Fusarone (**5**) was isolated as a colorless gum with the molecular formula $C_{14}H_{22}O_3$ by HREIMS. It showed absorption bands at 3209 and 1731 cm^{-1} for hydroxy and carbonyl groups, respectively. A 3,4-disubstituted cyclopentanone was established using a series of $^{1}H-^{1}H$ COSY cross-peaks (Supporting Information) from H_2-2 (δ_H 2.32 and 2.06) through H-3 (δ_H 1.82) to H-4 (δ_H 1.88) and then to H_2-5 (δ_H 2.16 and 1.42) as well as the HMBC correlations of both H_2-2 and H_2-5 with C-1 (δ_C 220.2). Another series of $^{1}H-^{1}H$ COSY correlations from H_2-6 (δ_H 2.35) through an olefinic proton (H-7, δ_H 5.23) to the other olefinic proton (H-8, δ_H 5.43) and then to H_2-9 (δ_H 2.01) and H_3-10 (δ_H 0.95) constructed a 2-pentenyl unit. The configuration of the double bond was assigned as *Z* due to a coupling constant of 10.8 Hz between H-7 and H-8. The $^{1}H-^{1}H$ COSY cross-peaks of H_2-12 (δ_H 1.75 and 1.63) and H_2-13 (δ_H 2.38) together with the HMBC correlations of both H_2-12 and H_2-13 to C-11 (δ_C 34.1) and C-14 (δ_C 177.8) established a 3-carboxypropyl unit. In the HMBC spectrum, H-3 of the cyclopentanone exhibited a ^{3}J HMBC correlation with C-7 (δ_C 125.3) of the 2-pentenyl unit (Supporting Information), while H_2-12 of the carboxypropyl unit showed the same correlation with C-4 (δ_C 40.9). These data sequentially connected the 2-pentenyl and the carboxypropyl units at C-3 (δ_C 54.8) and C-4 of the cyclopentanone ring. In the NOEDIFF experiment, irradiation of H_2-11 enhanced the signal intensity of H-3, but not any protons of the 2*Z*-pentenyl unit, indicating that two substituents on the cyclopentanone ring were *trans*-oriented. Therefore, fusarone (**5**) was identified as a new cyclopentanone derivative.

The isolated compounds **1**−**3**, **5**−**11**, **13**−**16**, **18**, and 2-(2′*S*-hydroxypropyl)-5-methyl-7-hydroxychromone were tested for antibacterial activity against *Staphylococcus aureus* (SA) and methicillin-resistant SA. Compounds **6**, **10**, **15**, **16**, and **18** exhibited very mild antibacterial activity against both strains (MIC values >350 μM). For antifungal activity, among the naphthoquinone derivatives **2**−**3**, **13**−**16**, and **18**, compounds **3** and **16** exhibited very weak antifungal activity against *Cryptococcus neoformans* and *Microsporum gypseum* (MIC values >200 μM).

The known octahydroanthraquinone **7** has been previously reported to exhibit antimalarial (against *Plasmodium falciparum* K1, IC_{50} 24.50 μM)[6] and antimycobacterial (against *Mycobacterium tuberculosis* H37Ra, MIC 38.57 μM)[6] activities. Thus, the anthraquinone (**6**, **7**, and **9**−**11**) and naphthoquinone (**2**, **13**, **15**, **16**, and **18**) derivatives were further evaluated for these activities (Table 4). Compounds **9**, **11**, **13**, and **18** displayed weak antimalarial activity, but better activity than **7**, with IC_{50} values in the range 9.8−14 μM. Compounds **11** and **18** exhibited milder activity than **7** against *M. tuberculosis*. In addition, they were evaluated for cytotoxic activities against oral human carcinoma cells (KB), human breast cancer cells (MCF-7), and noncancerous Vero cells (African green monkey kidney fibroblasts). Compounds **2**, **10**, and **15** were noncytotoxic to Vero cell lines. Compound **10** was selectively cytotoxic to MCF-7 cells, whereas compound **9** exhibited strong activity to all tested cell lines. Furthermore, compound **18** showed

Table 4. Antimalarial, Antimycobacterial, and Cytotoxic Activities of Some Metabolites

compound	antimalarial (IC_{50}, μM) *P. falciparum* K1	anti-TB (MIC μM) *M. tuberculosis*	cytotoxicity (IC_{50}, μM) KB	MCF-7	Vero
2	[d]	[d]	130	22	[d]
6	[d]	[d]	19	15	57
7	25	39	49	6.2	54
9	9.8	[d]	0.9	2.7	4.2
10	[d]	[d]	[d]	6.3	[d]
11	13	41	88	5.4	29
13	12	[d]	5.7	13	170
15	[d]	[d]	120	101	[d]
16	[d]	[d]	14	9.8	79
18	14	87	2.0	0.9	58
dihydroartemisinin[a]	0.004				
isoniazid[b]		0.17−0.34			
doxorubicin[c]			0.33	2.18	
ellipticine[c]			1.10		4.47

[a] Standard antimalarial drug. [b] Reference compounds for antimycobacterial activity. [c] Standard compounds for cytotoxicity assay. [d] Inactive.

significant selective cytotoxicity to both KB and MCF-7 cell lines. Interestingly, compounds **9** and **18** displayed cytotoxic activity as potent as the standard compounds against KB and MCF-7 cell lines, respectively. Because they share a common 1,4-naphthoquinone unit, this unit may play an important role in the activity. It is worth noting that the hydropyran unit in compound **18** might contribute to the selective cytotoxic activity.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO P-1020 polarimeter. The ultraviolet (UV) absorption spectra were measured in MeOH on a Shimadzu UV-160A spectrophotometer. The infrared (IR) spectra were recorded neat using a Perkin-Elmer 783 FTS165 FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a 300 MHz Bruker FTNMR Ultra Shield spectrometer. Chemical shifts are expressed in δ (ppm) referring to the tetramethylsilane peak. Mass data were obtained on a MAT 95 XL mass spectrometer (ThermoFinnigan). Thin-layer chromatography (TLC) and preparative TLC were performed on silica gel 60 GF_{254} (Merck). Column chromatography was carried out on Sephadex LH-20 with MeOH, silica gel (Merck) type 60 (230−400 mesh ASTM) or type 100 (70−230 mesh ASTM), or on reversed-phase silica gel C-18. Light petroleum had a bp of 40−60 °C.

Fungal Material. The marine-derived fungi *Fusarium* spp. PSU-F14 and PSU-F135 were isolated from a gorgonian sea fan (*Annella* sp.) collected near Koh Hin Ran Pet, Suratthani Province, on October 11, 2005, by Mr. Sakanan Plathong, the Department of Biology, Faculty of Science, Prince of Songkla University. They were deposited at the Department of Microbiology, Faculty of Science, Prince of Songkla University, as PSU-F14 and PSU-F135 (GenBank accession numbers EU714385 and EU714402, respectively) on October 20, 2005.

The fungus PSU-F135 was identified on the basis of its morphological and molecular characteristics. Its colony is rapid-growing, woolly to cottony, and white in color with some tinge of pink in the mycelium and pink to pale orange pigment in the agar medium. It produces two kinds of conidia: macroconidia with multiple cells, slightly curved at the pointed ends, and one-celled microconidia, which are the characteristics of the genus *Fusarium*.[19] Its 550 base pair ITS sequence (EU714402) had 98.2% sequence identity to that for *Fusarium* sp. NRRL 43815 (EF453208). This confirmed the identity of PSU-F135 to be a *Fusarium* sp. Colonies of the fungus PSU-F14 are also rapid-growing, velvety, and pale orange in color. They release a bright orange pigment into the agar medium. As neither conidia nor spores were observed, this fungus was identified on the basis of the analysis of the ITS regions of its rDNA gene. Its 525 base pair ITS sequence (EU714385) had 99.8% and 100% sequence identity to those for *Fusarium* spp. AF158314 and AF158313, respectively. This confirmed the identity of PSU-F14 to be a *Fusarium* sp.

Fermentation, Extraction, and Isolation. The marine-derived fungi *Fusarium* spp. PSU-F14 and PSU-F135 were grown on potato dextrose agar at 25 °C for 5 days. Three pieces (0.5 × 0.5 cm^2) of mycelial agar plugs were inoculated into 500 mL Erlenmeyer flasks containing 300 mL of potato dextrose broth at room temperature for four weeks. The flask culture (15 L) was filtered to separate filtrate from wet mycelia. The filtrate was divided into three portions. Each portion was extracted three times with an equal volume of EtOAc (3 × 800 mL). The combined EtOAc layer was dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure to give a dark red solid (3.7 g) and a red-brown gum (839.0 mg) from *Fusarium* spp. PSU-F14 and PSU-F135, respectively. The mycelia cake from the fungus PSU-F14 was extracted with MeOH (500 mL). The MeOH layer was concentrated under reduced pressure. H_2O (100 mL) was added to the extract, and the mixture was washed with hexane (300 mL). The aqueous residue was extracted three times with an equal volume of EtOAc (3 × 300 mL). The EtOAc layer was then dried over anhydrous Na_2SO_4 and evaporated to dryness to obtain a brown solid (560.0 mg). The extracts from the fungi PSU-F14 and PSU-F135 were then subjected to a variety of chromatographic techniques to give compounds **1** (3.1 mg), **2** (4.0 mg), **3** (3.0 mg), **4** (1.5 mg), and **5** (3.1 mg) and 18 other compounds. Details are shown in flow diagrams in the Supporting Information.

Fusaranthraquinone (1): colorless gum; $[\alpha]^{25}_D$ −18 (*c* 0.92, acetone); UV (MeOH) λ_{max} (log ε) 242 (3.91), 280 (3.61), 353 (3.42) nm; IR (neat) ν_{max} 3417, 1667 cm^{-1}; ^{1}H NMR (acetone-d_6, 300 MHz), see Table 1; ^{13}C NMR (acetone-d_6, 75 MHz), see Table 1; HREIMS *m*/*z* [M]$^+$ 324.1195 (calcd for $C_{16}H_{20}O_7$, 324.1209).

Fusarnaphthoquinone A (2): colorless gum; $[\alpha]^{24}_D$ −25 (*c* 0.45, MeOH); UV (MeOH) λ_{max} (log ε) 244 (3.51), 282 (3.69), 352 (3.26) nm; IR (neat) ν_{max} 3330, 1700, 1682 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 2; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 2; HREIMS *m*/*z* [M − H_2O]$^+$ 292.0948 (calcd for $C_{15}H_{16}O_6$, 292.0947).

Fusarnaphthoquinone B (3): red gum; $[\alpha]^{25}_D$ −11 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 227 (4.08), 312 (4.01), 360 (3.45) nm; IR (neat) ν_{max} 3425, 1629 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 2; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 2; HREIMS *m*/*z* [M]$^+$ 276.1001 (calcd for $C_{15}H_{16}O_5$, 276.0988).

Fusarnaphthoquinone C (4): red gum; $[\alpha]^{25}_D$ −125 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 227 (4.23), 275 (3.70), 296 (3.69), 403 (3.61), 473 (3.47), 500 (3.53), 535 (3.44), 571 (3.12) nm; IR (neat) ν_{max} 3470, 1717, 1644 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz), see Table 3; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 3; HREIMS *m*/*z* [M]$^+$ 550.1478 (calcd for $C_{29}H_{26}O_{11}$, 550.1475).

Fusarone (5): colorless gum; $[\alpha]^{23}_D$ −58 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 213 (3.18) nm; IR (neat) ν_{max} 3209, 1731 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.43 (1H, dtt, J = 10.8, 7.2, 1.5 Hz, H-8), 5.23 (1H, dtt, J = 10.8, 7.5, 1.5 Hz, H-7), 2.38 (2H, m, H-13), 2.35 (2H, m, H-6), 2.32 (1H, m, H-2a), 2.16 (1H, m, H-5a), 2.06 (1H, m, H-2b), 2.01 (2H, m, H-9), 1.88 (1H, m, H-4), 1.82 (1H, m, H-3), 1.77 (1H, m, H-11a), 1.75 (1H, m, H-12a), 1.63 (1H, m, H-12b), 1.42 (1H, m, H-5b), 1.31 (1H, m, H-11b), 0.95 (3H, t, J = 7.5 Hz, H-10); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 220.2 (C, C-1), 177.8 (C, C-14), 133.7 (CH, C-8), 125.3 (CH, C-7), 54.8 (CH, C-3), 40.9 (CH, C-4), 37.9 (CH_2, C-2), 34.1 (CH_2, C-11), 33.8 (CH_2, C-13), 26.9 (CH_2, C-5), 25.5 (CH_2, C-6), 22.3 (CH_2, C-12), 20.6 (CH_2, C-9), 14.1 (CH_3, C-10); HREIMS *m*/*z* [M]$^+$ 238.1560 (calcd for $C_{14}H_{22}O_3$, 238.1569).

9α-Hydroxydihydrodesoxybostrycin (6): $[\alpha]^{25}_D$ −8 (*c* 0.16, MeOH); [lit. $[\alpha]^{25}_D$ −5.6 (*c* 0.16, MeOH)].[6]

Antimicrobial Assays Using a Colorimetric Broth Microdilution Test. Antimicrobial activity was determined as described by the Clinical and Laboratory Standards Institute[20−22] and Drummond and Waigh.[23]

Antimycobacterial Assay. Antimycobacterial activity was determined against *M. tuberculosis* H37Ra using green fluorescent protein (GFP)-based fluorescent detection.[24]

Antimalarial Assay. The activity was evaluated against the parasite *P. falciparum* (K1, multi-drug-resistant strain), using the microculture radioisotope technique based on the method described.[25]

Cytotoxicity Assays. The activity assay against African green monkey kidney fibroblast (Vero) cells was performed in triplicate employing the method described by Hunt and co-workers.[26] The activities against KB and MCF-7 cell lines were evaluated using the resazurin microplate assay.[27]

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Supporting Information Available: Flow diagrams for the isolation of all metabolites, structures of all isolated metabolites, ^{1}H and ^{13}C NMR spectra of compounds **1**−**5**, and their 2D NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

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